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(54) Title: SCREENING METHOD FOR IDENTIFYING WOMEN AT INCREASED RISK FOR PRETERM DELIVERY		
(57) Abstract The present invention provides an early, biochemical indication of increased risk of preterm delivery. The method comprises obtaining a body fluid sample from a pregnant patient after about week 4 of gestation and determining the proportion of total human chorionic gonadotropin (hCG) in the sample that is in the intact form. A decreased proportion relative to that which is characteristic of pregnancies that proceed to term indicates an increased risk of preterm delivery.		

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SCREENING METHOD FOR IDENTIFYING WOMEN
AT INCREASED RISK FOR PRETERM DELIVERY

BACKGROUND OF THE INVENTION

5 Field of the Invention

This invention relates to methods for detection of increased risk of preterm delivery. In particular, this invention is directed to determining an early indication of increased risk of preterm delivery by determining the
10 proportion of human chorionic gonadotropin (hCG) that is in the intact form in a body fluid sample.

Description of the Prior Art

Determination of impending preterm births is critical for increasing neonatal survival of preterm infants. In
15 particular, preterm neonates account for more than half, and maybe as much as three-quarters of the morbidity and mortality of newborns without congenital anomalies. Although tocolytic agents which can delay delivery were introduced 20 to 30 years ago, there has been only a minor
20 decrease in the incidence of preterm delivery. It has been postulated that the failure to observe a larger reduction in the incidence of preterm births is due to errors in the diagnosis of preterm labor and to the patients' conditions being too advanced for tocolytic
25 agents to successfully delay the birth.

Traditional methods of diagnosis of preterm labor have high false-negative and false-positive error rates [Friedman et al, *Am. J. Obstet. Gynecol.* 104:544 (1969)]. In addition, traditional methods for determining impending
30 preterm delivery, particularly in patients with clinically intact membranes, may require subjective interpretation, may require sophisticated training or equipment [Garl et al, *Obstet. Gynecol.*

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60:297 (1982)] or may be invasive [Atlay et al, *Am. J. Obstet. Gynecol.* 108:933 (1970)]. Accordingly, an early, objective biochemical marker indicative of increased risk for preterm delivery was sought.

5 Recently, Lockwood et al [*New Engl. J. Med.* 325:669-674 (1991)] reported that fetal fibronectin in cervical and vaginal secretions indicates pregnancies which are at risk of imminent delivery. The authors postulate that damage to the fetal membranes may release
10 fetal fibronectin into the cervix and vagina, thus giving rise to the biochemical marker.

Other markers which may be released in women with true threatened pregnancies can be used to screen those women who should be closely monitored and to provide
15 additional information about the stage of the disease.

SUMMARY OF THE INVENTION

The present invention provides an early, biochemical indication of increased risk of preterm delivery. The method comprises obtaining a body fluid sample from a
20 pregnant patient after about week 4 of gestation and determining the proportion of human chorionic gonadotropin (hCG) that is in the intact form in the sample. A decreased proportion relative to that which is characteristic of pregnancies that proceed to term
25 indicates an increased risk of preterm delivery. The test is preferably administered to women at about 4 weeks gestation and repeated at each prenatal visit (every two to four weeks) until at least week 37, preferably until delivery if the test is negative. For those patients
30 whose assay result indicates an increased risk of preterm delivery, a test of the patient's fetal fibronectin level can be made to confirm the increased risk and to estimate how soon the delivery will be. In addition, those patients can be carefully monitored, as for other patients
35 at risk.

The test is both a sensitive and specific screen for

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pregnancies at risk and can detect an increased risk of preterm delivery as early as two to four weeks prior to delivery. The method not only allows early intervention in the course of preterm delivery but also provides an additional factor which can indicate those pregnancies at greatest risk.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is a screening assay which provides an early, biochemical indication of increased risk of preterm delivery based on detection of a decreased proportion of intact human chorionic gonadotropin (hCG) in a body fluid sample. The method can provide an indication of impending delivery as early as two to three weeks prior to delivery. This method allows early intervention in the course of preterm delivery and provides an additional factor which can indicate those pregnancies at greatest risk.

The method comprises obtaining a body fluid sample, preferably serum or urine, from a pregnant patient after about week 4 of pregnancy and prior to about week 36 or 37, and determining the proportion of human chorionic gonadotropin (hCG) that is in the intact form. A decreased proportion relative to that which is characteristic of pregnancies that proceed to term indicates a patient who is at risk for preterm delivery. In a preferred embodiment, the proportion of hCG that is in the intact form is determined using an immunoassay. Since inflammatory conditions in the local membranes could damage trophoblast secreted proteins such as hCG, the amount of intact hCG in the local area as reflected in cervicovaginal secretion samples, as well as the amount systemically (e.g. in blood and urine samples) can be used as an indicator of the damage at the maternal fetal interface.

The present invention can determine increased risk of preterm delivery between weeks 4 and 37 of gestation.

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Deliveries between 4 to 20 weeks gestation are generally termed spontaneous abortions rather than preterm deliveries. Term pregnancies are from 37 to 40 weeks.

Intact and Nicked Human Chorionic Gonadotropin (hCG)

5 The present invention is based on detection of a significant decrease in the proportion of intact hCG that occurs in body fluids of women who deliver preterm. Total hCG is the sum of nicked hCG and intact hCG. The proportion of intact hCG can be determined using any
10 method that determines the relative amounts of any two of total hCG, nicked hCG, and intact hCG.

hCG is a glycoprotein hormone produced almost exclusively by the placenta. The polypeptide portion of hCG is a dimer that is composed of an alpha subunit (92
15 amino acid residues) and a beta subunit (145 amino acid residues), joined noncovalently. The beta chain contains a disulfide bridge between cysteines 38 and 57. The appearance of hCG in patient urine is currently the most commonly used indicator to determine pregnancy.

20 hCG is found in the blood and urine of pregnant women as a mixture of two forms: an intact form and a proteolytically nicked form. The nicked form is the same as the intact form but for a single break in the beta-subunit polypeptide chain between either residues 44
25 and 45, residues 47 and 48, or far less commonly, residues 46 and 47. The nicked form comprises, on average, about one quarter of the total hCG population in both the blood and urine of pregnant women who deliver at term.

The alpha- and beta-chains of hCG are also present as
30 free subunits. In addition, a fragment of the beta-chain, called the beta-core fragment, which comprises beta-chain residues 6-40 disulfide-linked to beta-chain residues 55-92, may also be present.

Other known variants of hCG include truncated forms
35 which lack the first two or three N-terminal amino acids of the alpha chain. However, these truncated forms are

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characteristic of relatively rare pregnancy-associated cancers (hydatidiform mole and choriocarcinoma) and are not observed in normal pregnancies.

Patients to be Tested

5 The present method can be used on any pregnant woman following about 4 weeks gestation and prior to term (week 36 or 37). In addition to screening any pregnant woman to determine whether the patient is at risk for preterm delivery, the patients who are preferably screened are
10 those patients with clinically intact membranes in a high risk category for preterm delivery, and more preferably, all those women whose pregnancies are not sufficiently advanced to ensure delivery of a healthy fetus. Ninety percent of the fetal morbidity and 100 percent of the
15 fetal mortality associated with preterm delivery is for those fetuses delivered prior to 32 to 34 weeks gestation. Therefore, 32 to 34 weeks gestation is an important cutoff for the health of the fetus, and preferably women whose pregnancies are at least about 4 weeks and prior to 34
20 weeks in gestation are tested.

In addition there are a large number of factors known to be associated with the risk of preterm delivery. Those factors include multiple fetus gestations, incomplete cervix, uterine anomalies, polyhydramnios, nulliparity,
25 previous preterm rupture of membranes or preterm labor, preeclampsia, first trimester vaginal bleeding, little or no antenatal care, and symptoms such as abdominal pain, low backache, passage of cervical mucus, and contractions. Any pregnant woman at 4 or more weeks gestation with
30 clinically intact membranes and having one or more risk factors for preterm delivery is preferably tested throughout the risk period; i.e., until about week 34 to 37.

The Sample

35 The sample is a body fluid sample, preferably blood,

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urine or cervicovaginal secretions, and is collected according to standard procedures. A blood sample can be a plasma or, preferably, a serum sample. The sample is preferably frozen following processing if the sample cannot be analyzed within a few hours of collection. The urine sample can be a random sample, preferably a first morning specimen, or more preferably, a 24 hour sample. Urine samples which are not assayed within 24 hours of collection are preferably stored at 4°C, and more preferably, are stored frozen. A cervicovaginal secretion sample is generally obtained from the vaginal cavity or the external cervical canal using a swab having an absorbent material; e.g., cotton or dacron.

Assay Procedure

As stated previously, the proportion of intact hCG can be determined using any method that determines the relative amounts of any two of total hCG, nicked hCG, and intact hCG. Immunoassays that quantitate total and intact hCG are preferred. However, non-quantitative assays that determine relative amounts of nicked and intact hCG or total and intact hCG in a given volume of sample can also be used.

Total hCG is the sum of the nicked and intact forms of hCG. The proportion of intact hCG is the amount of intact hCG divided by the amount of total hCG. Therefore, when the proportion of hCG that is nicked is known, the proportion that is intact is the difference of the total hCG minus the nicked proportion. Similarly, when the relative levels of nicked and intact hCG are known, the proportion of hCG that is intact is the level of intact hCG divided by the sum of the levels of the intact and nicked forms. Therefore, determining the proportion of nicked hCG is equivalent to determining the proportion of intact hCG. Preferably, the proportion of intact hCG is reported as a percentage.

The proportion of intact hCG is preferably determined

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by quantitating intact hCG and total hCG using an immunoassay. Alternatively, assays that determine the relative amounts of proteins in a sample, such as Western blot assays, can be used to determine the relative amounts of intact hCG and nicked hCG to determine the proportion of intact hCG. Determining the proportion of total hCG that is in the intact form requires not only distinguishing nicked from intact hCG, but also distinguishing nicked and intact hCG from the free alpha- and free beta-chains.

Antibodies specific for hCG and its subunit chains are well known. At least two epitopes have been reported on the alpha-chain and at least three on the beta-chain. In addition to the epitopes on each of the chains, there is an epitope that is present on the intact hCG dimer which is not present on either of the free chains or on nicked hCG. See, for example, Krichevsky et al [Endocrinology 123:584-593 (1988)] and the references cited therein, which describe various hCG epitopes. That article and the references cited therein are hereby incorporated by reference in their entireties.

Anti-hCG antibodies can be polyclonal or monoclonal for the purposes of the present invention and can be produced and purified by conventional methods. Such methods are described in a number of publications including Tijssen, P. Laboratory Techniques in Biochemistry and Molecular Biology: Practice and Theories of Enzyme Immunoassays New York: Elsevier (1985).

In addition, antibodies to hCG are available. For example, a polyclonal antibody specific for the beta-chain is commercially available (Bios Pacific, Inc., Emeryville, CA). Anti-alpha-chain antibodies are available from Unipath (Cambridge, U.K.). In addition, a monoclonal antibody, designated B109, that is specific for the hCG dimer [described in Krichevsky et al, Endocrinology 123:584-593 (1988); Cole et al, Endocrinology

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129:1559-1567 (1991)] is used at numerous centers in the U.S. to detect low levels of hCG. This antibody does not recognize free alpha- or free beta-chains or nicked hCG and, therefore, can be used to distinguish intact hCG from
5 nicked hCG.

At present, there is no known antibody specific for the nicked form of hCG. However, if an epitope is identified that is present on the nicked form that is not present on the intact form, the immunoassay can quantitate
10 nicked hCG and total hCG or quantitate nicked hCG and intact hCG to determine the proportion of intact hCG in the sample.

A number of different types of immunoassays are well known using a variety of protocols and labels. The assay
15 conditions and reagents may be any of a variety found in the prior art. The assay can be heterogeneous or homogeneous and is conveniently a sandwich assay.

The assay usually employs solid phase-affixed antibodies. The solid phase-affixed antibodies are
20 combined with the sample. Binding between the antibodies and sample can be determined in a number of ways. Complex formation can be determined by use of soluble antibodies. The soluble antibodies can be labeled directly or can be detected using labeled second antibodies specific for the
25 species of the soluble antibodies. Various labels include radionuclides, enzymes, fluorescers, colloidal metals or the like. Conveniently, the assay will be a quantitative enzyme-linked immunosorbent assay (ELISA) in which antibodies specific for hCG are used as the solid
30 phase-affixed antibodies and enzyme-labeled, soluble antibodies.

To assay total hCG, the assay can use any two hCG antibodies for hCG that do not compete for a binding site, with the exception of dimer-specific antibodies.
35 Preferably, the assay uses an antibody for the alpha-chain and an antibody for the beta-chain to avoid potential quantitation of either of the free chains. However, when

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the relative amount of the free chains is not a significant proportion of total hCG, the assay can use two antibodies to the same chain. To assay intact hCG, the assay can use a dimer-specific antibody together with an antibody for the alpha-chain or, preferably, the beta-chain.

Alternatively, the assay can be based on competitive inhibition, where analyte in the sample competes with a known amount of analyte or analyte analog for a predetermined amount of anti-analyte antibody. In the competitive format, a dimer-specific antibody is used to detect intact hCG and an antibody for either the alpha-chain or the beta-chain is used to quantitate total hCG. When the relative amount of the free chains are not a significant proportion of the total hCG, the free chains do not affect the assay result. However, when the relative amounts of the free chains are significant, preferably an assay using an antibody for the alpha-chain and an antibody for the beta-chain such as a sandwich assay, rather than competitive assay, is used.

A standard with a known amount of intact hCG is used. Preferably, the hCG in the standard is at least 80% intact. Standards with known amounts of intact hCG are available commercially. As recently reported, the Columbia anti-hCG test that has been used to quantitate total hCG in some hCG standards does not detect nicked hCG [Birken et al, *Endocrinology* 129:1551-1558 (1991); Cole et al, *Endocrinology* 129:1559-1567 (1991)]. Therefore, the hCG standard is preferably one in which the proportion of intact hCG has been determined by amino acid sequence analysis.

Other quantitative methods to determine the proportion of hCG that is in the intact form can be envisaged. For example, a method to quantitate total and nicked hCG using amino acid sequence analysis of hCG purified from urine has been described [Khardana et al, *Endocrinology* 129:1541-1550 (1991)]. An exemplary

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procedure to determine the proportion of hCG that is intact in a serum or urine sample by quantitating total and intact hCG is described in detail in the Examples.

In addition to methods that quantitate hCG forms, any method that determines relative amounts of total and intact hCG can also be used. For example, a Western blot assay can be used to determine the relative amounts of total and intact hCG by determining the relative amounts of nicked and intact hCG beta-chains. Briefly, the electrophoresis is performed under denaturing conditions which separate the alpha-chain from the beta-chain. A reducing agent, for example β -mercaptoethanol, is used to reduce disulfide bonds in the beta-chains. Following treatment with a reducing agent, the beta-chain of intact hCG remains a single chain with an apparent molecular weight of about 34 to 37 kD and the beta-chain of nicked hCG separates into an N-terminal fragment of about 17 kD and a C-terminal fragment of about 24 kD, as determined by electrophoresis.

Following electrophoresis, the separated proteins are transferred to a support membrane and detected with labeled anti-beta C-terminus antibody. The antibodies react with the intact beta-chain (34-37 kD) and the nicked beta-chain C-terminal fragment (24 kD). The size differences between the intact and nicked chains facilitate distinguishing the intact and nicked beta-chains. The relative amounts of intact and nicked beta-chain are determined by appropriate methods, depending on the label on the antibody. The proportion of hCG in the intact form is the relative amount of intact beta-chain divided by the sum of intact beta-chain and nicked beta-chain C-terminal fragment. Preferably, the original sample is also tested for free, intact beta-chain to ensure that the level of intact hCG is not overestimated.

A Western blot method to determine the proportion of hCG that is in the intact form is described in Cole et al

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[supra]. Other methods which measure relative amounts of total, nicked, and intact hCG can also be envisaged.

Interpretation of Result

A decrease in the proportion of hCG that is in the intact form relative to the proportion that is characteristic of pregnancies that proceed to term indicates an increased risk of preterm delivery. Preferably, the threshold value that separates risk from non-risk cases is two standard deviations below the average value for pregnancies that proceed to term. A preferred threshold below which the patient is considered to be at risk for preterm deliver is 25% or less intact hCG (as a fraction of total hCG in the sample). A more preferred value is 10% or less intact hCG. As is well known, the 25% threshold value will detect more false positive values. However, a somewhat high false positive rate is acceptable in a screening assay, where the objective is to detect all those at risk. For an assay which has a lower false positive rate, but which has a higher false negative rate, the lower threshold is selected. Since the proportion of intact hCG can also be expressed as the proportion of nicked hCG, or as the ratio of nicked to intact hCG, thresholds appropriate for such expressions can also be used. However, since the proportion of total hCG that is in the intact form varies considerably among women with pregnancies that proceed to term, it is preferable that patients with samples near the threshold value be retested in a follow up visit.

If the hCG test is positive (i.e., the proportion of hCG in the intact form is below the threshold value), the patient is preferably tested for the presence of fetal fibronectin in her cervicovaginal secretions. If fetal fibronectin is present in the secretions, the patient is likely to deliver in two to three days. Measures to determine or enhance fetal lung maturity can be undertaken. If the fetal fibronectin assay is negative,

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the patient should be carefully monitored and repeated evaluations of the patient's fetal fibronectin levels should be performed on subsequent visits. In general, patients at risk for preterm delivery are examined every 5 two weeks from about 22 to 36 weeks, rather than every four weeks as for patients in a low risk category.

If the hCG test is negative (the proportion of hCG in the intact form is above the threshold), the test is preferably repeated on each subsequent visit until either 10 the test is positive or the patient reaches term.

The procedure is sensitive and specific. Since the test successfully detects a large percentage of patients who deliver early, the test is an effective screening procedure for women at risk for preterm delivery who do 15 not have any other risk indicators.

This invention is further illustrated by the following specific but non-limiting examples. Temperatures are given in degrees Centigrade and concentrations as weight percent unless otherwise 20 specified. Procedures which are constructively reduced to practice are described in the present tense, and procedures which have been carried out in the laboratory are set forth in the past tense.

EXAMPLE 1

25 The following immunoassay method determines the proportion of total hCG that is in the intact form. The method can be used for a serum or urine sample. The proportion of total hCG that is intact is determined by measuring the concentrations of intact hCG and total hCG 30 by separate assays.

The concentration of intact hCG in the sample is determined using a B109:anti- β -peroxidase assay performed as described by Cole et al [*Endocrinology* 129:1559-1567 (1991)]. This immunoassay uses a hCG dimer-specific 35 monoclonal antibody designated B109 (available from Drs. A. Krichevsky and E. Armstrong of Columbia University) as

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the solid phase-affixed antibody to capture intact hCG, and peroxidase-labeled, goat anti-beta-chain antiserum (Bios Pacific, Inc., Emeryville, CA) to detect bound intact hCG. Microtiter plates are coated with antibody B109 (200 μ l; 2 μ g/ml in 0.25 M sodium carbonate, pH 9.5, containing 0.1 M NaCl) and plates are incubated overnight at 4°C. Plates are washed five times with water and aspirated before use.

In triplicate, sample or purified, intact hCG standard (100 μ l) is added to coated wells together with buffer-carrier protein mix (100 μ l; 0.05 M sodium phosphate, pH 7.5, containing 0.14 M NaCl and 0.1% ovalbumin). hCG standard solutions (0, 2.5, 5, 10, 15, 20, and 25 ng intact hCG/ml) are used to establish a standard curve.

The plates are shaken on a plate rotator overnight at ambient temperature and then washed five times with water and aspirated. Peroxidase-labeled goat anti-beta-chain antiserum (200 μ l; 1:3500 dilution in 0.1 M Tris-HCl, pH 7.5, containing 0.025 M CaCl₂ and 0.1% ovalbumin) is then added to the wells, and the plates are shaken for 2 hours at ambient temperature. After another five washes with water, 200 μ l of substrate mix is added (prepared by the addition of a 5 mg tablet of orthophenylenediamine [Sigma Chemical Company] and 4 μ l 30% H₂O₂ to 25 ml of 0.01 M sodium citrate, pH 4.9), and the plates are shaken in the dark for 30 min at ambient temperature. Hydrochloric acid (50 μ l; 4 M) is added to stop the reactions, and the absorbance of the wells is determined in a TITERTEK MULTISCAN NCC-340 plate reader (Flow Laboratories, McClean, VA) at 492 nm. Data are sent to a Zenith 80286 computer, and standard curves are plotted and levels determined using TITERSOFT software (Flow Laboratories). All values are determined in triplicate. This assay is specific for intact hCG, with less than 1% cross-reactivity with free beta-subunit, free alpha-subunit, and human luteinizing hormone (hLH).

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Total hCG is quantitated in the same way except that a monoclonal anti-alpha-chain antibody (Unipath) 4 μ g/ml in the microtiter plate coating step) is used in place of the B109 antibody, a change that renders the assay equally specific for intact and nicked hCG. This assay is specific for total hCG, with less than 0.1% cross-reactivity with hCG free beta-subunit, free alpha-subunit, or hLH.

EXAMPLE 2

10 A serum sample from a pregnant women at 26 weeks gestation is assayed for intact and total hCG according to the procedure in Example 1. The proportion of hCG that is in the intact form in the sample is found to be 10%. This value indicates that the woman is at increased risk for
15 preterm delivery.

EXAMPLE 3

A cervicovaginal secretion sample from a pregnant women at 26 weeks gestation is assayed for intact and total hCG according to the procedure in Example 1. The
20 proportion of hCG that is in the intact form in the sample is found to be 75%. This value indicates that the woman is not at increased risk for preterm delivery.

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WHAT IS CLAIMED IS:

1. A method for determining an early indication of increased risk of preterm delivery comprising
 - 5 a. obtaining a body fluid sample from a pregnant patient after week 4 and before week 37 of pregnancy; and
 - b. determining the proportion of total human chorionic gonadotropin that is in the intact form in the sample, a decreased proportion
10 relative to that which is characteristic of pregnancies that proceed to term indicating an increased risk of preterm delivery.
2. The method of Claim 1 wherein the sample is a blood sample.
- 15 3. The method of Claim 2 wherein the sample is a serum sample.
4. The method of Claim 1 wherein the sample is a urine sample.
5. The method of Claim 1 wherein the proportion is
20 determined by a non-quantitative assay.
6. The method of Claim 1 wherein the proportion is determined by a quantitative assay.
7. The method of Claim 6 wherein the level of total human chorionic gonadotropin and the level of intact
25 human chorionic gonadotropin are quantitated using an immunoassay.
8. The method of Claim 7 wherein the immunoassay is a sandwich immunoassay.
9. The method of Claim 7 wherein the immunoassay is a
30 competitive immunoassay.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/04735

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : G01N 33/566, 33/567, 33/53; C12Q 1/00

US CL : 436/501, 503, 510; 435/7.1, 7.92, 7.93, 7.94, 7.95

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/501, 503, 510; 435/7.1, 7.92, 7.93, 7.94, 7.95

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Medline, Biosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HENRY, "Clinical Diagnosis and Management by Laboratory Methods," published 1979 by W.B. Saunders Company (Philadelphia), see pages 680-692, see especially Figure 19 and pages 685-686.	1-9
Y	US, A, 4,310,455 (BAHL) 12 JANUARY 1982, see column 1, line 65 and column 12, line 42.	1-9
Y	US, A, 4,954,434 (MOROZ) 04 SEPTEMBER 1990, see column 5, line 31 and column 4, line 54.	1-9

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 25 June 1993	Date of mailing of the international search report 14 JUL 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer DAVID R. PRESTON Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/04735

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Endocrinology, Volume 129, No. 3, issued March 1991, L.A. Cole et al., "The heterogeneity of human chorionic gonadotropin (hCG). III. The occurrence and biological and immunological activities of nicked hCG," pages 1559-1567, see especially page 1562, paragraph 1, right; page 1563, paragraph 2, left; page 1565, line 26, right; and page 1562, results section.	1-9